Analysis of isoperoxidases of *Populus* spp. in Heilongjiang Province

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Abstract Isoperoxidases of fourteen species of poplars in Heilongjiang Province were analyzed by polyacrylamidegel disc-electrophoresis. The major zymogram of petioles of poplars was consisted of twelve kinds of isoenzymes, in which 35% zymogram was just the same and represented the specialized zymograms of poplars. The results of the experiments showed that the closer the affinity relationships of the species were, the greater the similar values between the zymograms were. There were very great similar values among natural hybrid species. But in sect. *Tacamahaca* Spach, the similar values between *Populus koreana* Rehder and *P. nakaii* Skv. were obviously smaller. Isoenzyme analysis provided new information for plant classification on the molecular level.

Key words: Poplar, Isoperoxidases, Polyacrylamidegel disc-electrophoresis

Introduction

Biochemical analysis about various organs of different plants, in different grown stages and habitats, has become an important ways for providing new characteristics and new information in plant classification. Isoenzyme is a kind of zymes existed in many different tissue cells, of which structures are not exactly same, but can catalyze a same kinds of chemical reaction. As is known to all, the formation of zyme is controlled by one or more genes, so it is a direct product of gene expression and can be taken as a biochemical target of gene expression. As the species in evolution process may be influenced by rebuilding and mutation of genes, the varied gene compositions can reflect on the isozymogram and form the specificity of genus and species in isozymogram. It is an effective method to study the plant relationship using plant isoenzyme spectums (Smith 1980; Smith 1986).

Isoperoxidase is a kind of zymes which can use H_2O_2 oxidizing hydrogeno, and it is very specialized in requiring H_2O_2, but is wider in requiring hydrogeno. Isoperoxidase exists widely and largely among high plants, and is a necessary zyme for light respiration, so more researches have been done about it. Hu Zhiang *et al.* studied the biological phylogenesis of *Populus* spp. and *Hovenia* spp. using isoperoxidase. Jiang Hong *et al.* studied the relationship of *Cupressus* L. using isoperoxidase (Jiang 1986). Zhong Huiwen and Li Chunzheng *et al.* individually identified the sex of *Ginkgo biloba* and *Actinidia chinensis* Planh using isoperoxidase (Zhong 1982; Li 1986).

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We take the regional local *Populus* spp. as main targets to analyze their isoperoxidase and find the relationship between them for providing taxonomy with new messages.

Materials and methods

Samples

The samples of *Populus* spp. (Table 1) were picked up from the last ten days of August to the first ten days of September in 1986, and stored in refrigerator (-20°C). According to the reports of Mr. Hu Zhi'ang, the branches and mature petioles of *Populus* spp. had the same zymogram that did not change by season. Sex has not obvious effects on zymogram. and the isozymograms of the petioles obtained from different locations are very similar. So the samples, except those of germinated leaves, can be picked up at random.

The preparation of sample liquid

Refrigerated petioles (1 g) are put in a mortar settled in ice and water. 0.1 mol/L phosphoric acid buffer (1 mL, pH=5) is put into the mortar, then the phosphoric acid is polished into paste, and centrifugalized 15 min with the speed of 3 000 rounds/min. The above flesh liquid is the sample.

Measuring isoenzymes

Polycylamidegel disc-electrophoresis and anode zymes were used for measuring isoenzymes (Huang 1980). The zymogram was dyed with Benzidine acetic acid and scanned with C-S 910 scanner, λ_0=800 nm, λ =400 nm.

Table 1. The species name and origin of *Populus* spp.

Species	Materials origin
Populus nakaii Skv.	Botanical Garden of Heilongjiang Province
P. maximowiczii Henry .	Harbin Forest Farm of Northeast Forestry University
P. simonii Carr.	Harbin Forest Farm of Northeast Forestry University
P. koreana Rehder.	Botanical Garden of Heilongjiang Province
P. suaveolens Fischer .	Da Xing'an Mountains Area
P. alba L.	Campus of Northeast Forestry University
P. harbinensis Wang. et Skv.	Campus of the Chinese Medical University of Heilongjiang Province
P. ussurieusis Kom.	Botanical Garden of Heilongjiang Province
P. pseudo-simonii Kitag	Campus of Northeast Forestry University
P. canadensis Moench.	Campus of Northeast Forestry University
P. davidiana Dode.	Harbin Forest Farm of Northeast Forestry University
P. berrolinensis Dippol.	The Campus of Northeast Forestry University
P. nigra L.	Botanical Garden of Heilongjiang Province
P. pyramidalis Rozier.	Campus of Northeast Forestry University

Results

The analysis of isozymograms of *Populus* spp. (Fig. 1).

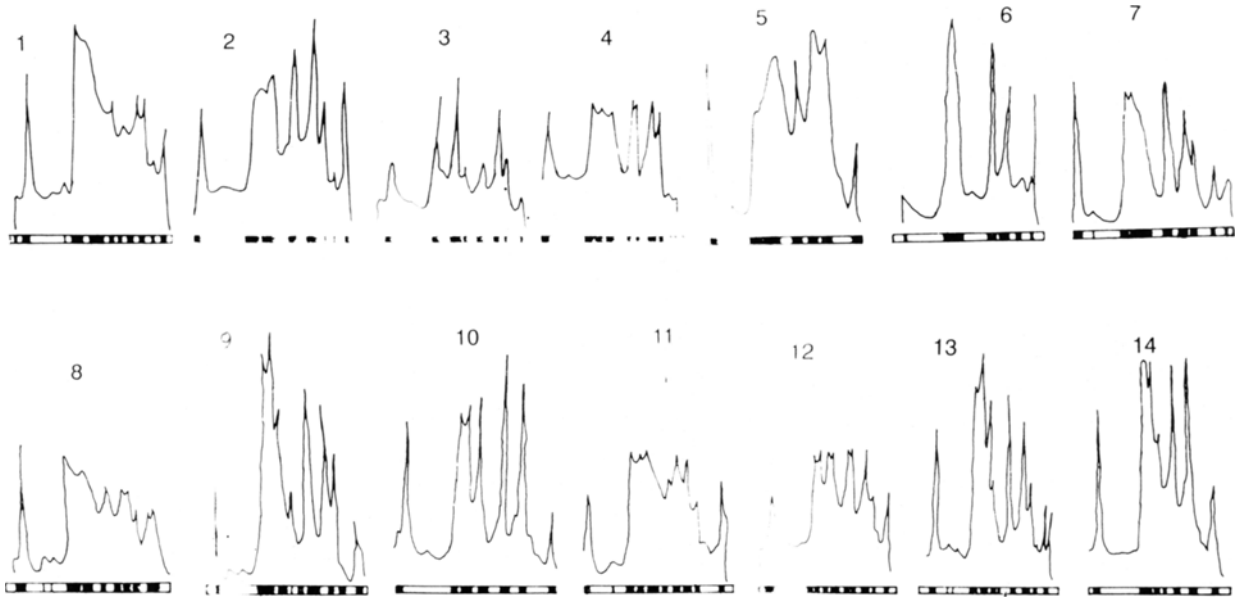

Fig. 1. Scanning graph of analysis of isoenzymes zymograms of *Populus* spp.

1. *Populus nakaii* Skv.,
2. *P. maximowiczii* Henry,
3. *P. simonii* Carr.,
4. *P. koreana* Rehder,
5. *P. suaveolens* Fischer.,
6. *P. alba* L.,
7. *P. harbinensis* Wang. et Skv.,
8. *P. ussuriensis* Kom.,
9. *P. pseudo-simonii* Kitag.,
10. *P. canadensis* Moench,
11. *P. davidiana* Dode.,
12. *P. berrolinensis* Dippol.,
13. *P. nigra* L.,
14. *P. pyramidalis* Rozier.

The measurement of similarity of the isoenzymes of *Populus* spp.

The kinds of zymes with the same R_f value (Table 2) under the same conditions are regarded as the same zymes. The similar coefficient among tree species, which represent their similar degree, is calculated. Similar index $C=2w/(a+b)\times 100\%$, a and b are the numbers of bands of zymogram A and B respectively. w is the numbers of the same of zymogram A and B.

The similar index (C) is below the line (Table 3). Non-similar value ($100-C$) is above the same line. The tesucts shows their similar degree among zymograms (Table 3).

Table 2. R_f values of isoperoxidases of *Populus* spp.

Species	Number of zymes band											
	1	2	3	4	5	6	7	8	8	10	11	12
Populus nakaii	0.02	0.01		0.20		0.32		0.38			0.58	
P. simonii	0.02		0.14	0.20		0.32	0.36		0.46			0.60
P. maximowiczii	0.02	0.07	0.15	0.20			0.34			0.53		0.63
P. koreana			0.13	0.19		0.33				0.50		
P. harbinensis	0.02		0.15	0.20			0.34				0.50	
P. suaveolens	0.02		0.14	0.22			0.35					0.60
P. pseudo-simonii	0.02		0.15	0.20			0.34		0.45			0.60
P. ussuriensis	0.02	0.10	0.15	0.21			0.35				0.58	
P. davidiana	0.02			0.19	0.25	0.33		0.39				0.60
P. alba	0.02	0.09		0.20	0.26	0.32						0.60
P. pyramidalis	0.02			0.20		0.32				0.52		
P. canadensis	0.02			0.20			0.35			0.51		0.62
P. nigra	0.02		0.15	0.21		0.33					0.56	
P. berrolinensis	0.02		0.14	0.20			0.35			0.51		0.62

Table 3. Similar and non-similar coefficient rectangular matrix table

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
A		54	54	60	45	64	67	50	50	67	40	64	27	67
B	46		29	45	3	17	8	38	38	38	45	33	50	23
C	46	71		45	33	17	23	23	54	38	45	17	67	54
D	40	55	55		56	60	60	60	60	60	25	56	33	40
E	55	67	67	44		20	27	9	64	64	56	40	40	27
F	36	83	83	44	80		9	27	45	5	56	20	40	9
G	33	92	77	40	73	91		33	50	50	60	27	64	17
H	50	62	77	40	91	73	67		67	50	60	64	45	33
I	50	62	46	40	36	55	50	33		17	40	45	45	50
J	33	62	62	40	36	55	50	50	83		40	45	45	50
K	60	55	55	75	44	44	40	40	60	60		33	33	40
L	36	67	8	44	60	80	73	36	55	55	67		60	9
M	73	50	33	67	60	60	36	55	55	55	67	40		45
N	33	77	46	60	73	91	83	67	50	50	60	91	55	

Note: A--*Populus nakaii*, B--*P. simonii*, C--*P. maximowiczii*, D--*P. koreana*, E--*P. harbinensis*, F--*P. suaveolens*, G--*P. pseado-simonii*, H--*P. ussuriensis*, I--*P. davidiana*, J--*P. alba*, K--*P. pyramidalis*, L--*P. canadensis*, M--*P. nigra*, N--*P. berrolinensis*

Discussion

Characteristics of zymograms of *Populus* spp.

The zymograms are similar (Fig. 1), every analyzed species has band 4, which is a special band for *Populus* spp. Except *P. koreana*, and other species has band 1, whose appearing rate is 92%. 35% of the isoenzymes spectrum detected in those *Populus* spp. are the same. But 12 kinds of isoenzymes lead to the formation of special zymograms of different species. According to Mr. Wang Zhan and Mr. Dong Shilin, there is difference in zymograms between groups, and the zymograms tend to be similar in a group. For example, the similar value among *P. simonii, P. pseudo-simonii, P. suaveoiens, P. harbinensis, P. maximowiczii* and *P. ussuriensis* is more than 60. The

similar value of *P. koreana* and *P. nakaii* is less than that of the other species in the same group. The zymograms of this group were concentrated on No. 1, 3, 4 and 7 strip, which formed the main band in this group. Band 5 is special for *P. davidiana* and *P. alba*, the similar value between them is 83.

The affinity between peroxide zymes and species

Zymes is the kind of protein having catalytic activity, and the expressible style of genetic information. The closer the affinity of the species is, the greater the similar values between the zymograms are. Many experts show that *P. pseudo-simonii* is a hybrid of *P. simonii* and *P. koreana*, and the zymogram of the hybrid is the addition of its parents, the similar value between them is 92. *Populus harbinensis* is the hybrid of *P. simonii* and *P. berrolinensis*. The enzyme similar value of *P. harbinensis* and *P. simonii* is 67, and the value of *P. harbinensis* and *P. berrolinensis* is 73. In addition, The similar values between *P. ussuriensis* and *P. harbinensis, P. pseudo-simonii* and *P. suaveolens, P. berrolinensis, P. berrolinensis* and *P. canadensis* are more than 90.

The significance of isoenzymes in taxonomy of poplar

The peroxide zymes in the petioles and trunks of *Populus species* are stable. The isoenzymes is in taxonomy. Electrophoresis, scanning and calculating can provide valuable data, which leads the level of shape description to the level of molecule analysis in the field botany taxonomy. So the isoenzymes analysis of the zymes is the reliable information of poplar taxonomy

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